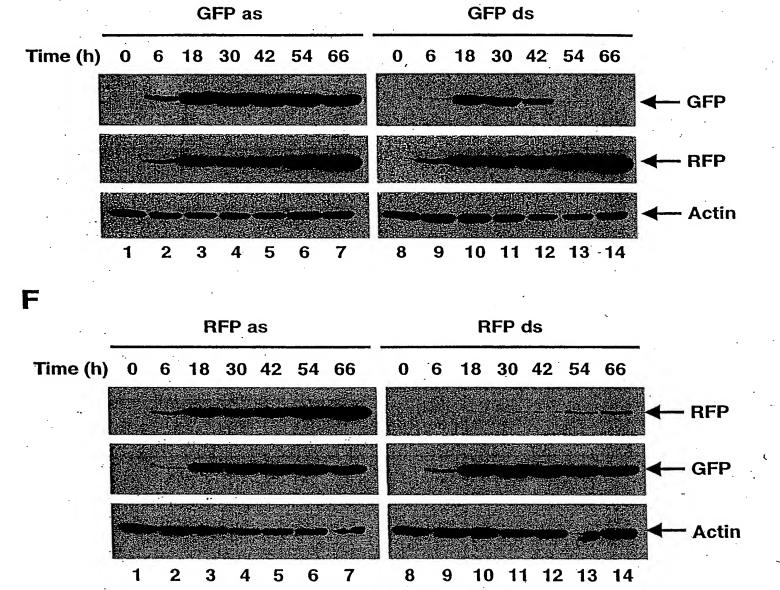


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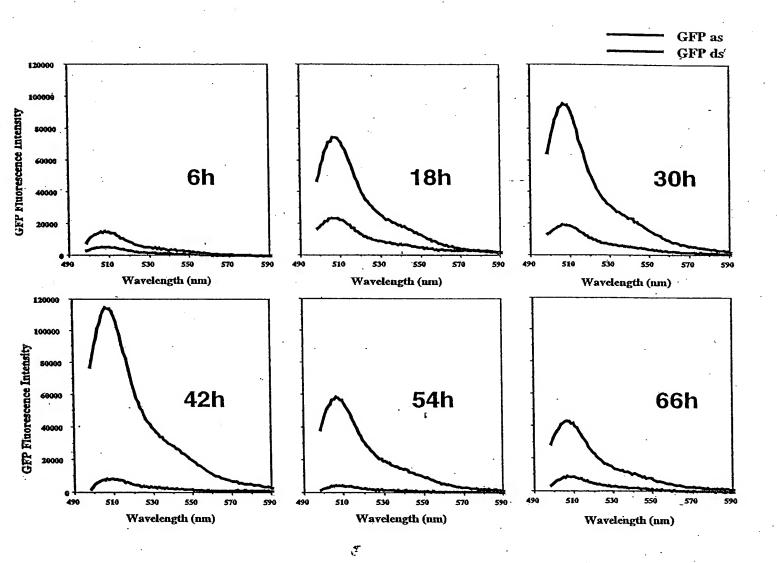
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Figure 1G



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NHE 3'- Puromycin = Pmn

5'- Amino modification = N3

B

	ds (WT)	GCAGCACGACUUCUUCAAGdTdT
		dtdtcgucgugcaaaaaguuc
5' Modified siRNA	5´-N3ss/as	N3 - GCAGCACGACUUCUUCAAGdTdT
		dTdTCGUCGUGCUGAAGAAGUUC
	ss/5´-N3as	GCAGCACGACUUCUCAAGdTdT
		dtdtcgucgugcugaagaaguuc - N3
	5'-N3ss/5'-N3as	N3 - GCAGCACGACUUCUUCAAGdTdT
		dtdtcgucgugaagaaguuc - N3
3' Modified siRNA	ss3´-Pmn/as	GCAGCACGACUUCUUCAAGdTdT - Pmn dTdTCGUCGUGCUGAAGAAGUUC
	ss/as3′-Pmn	GCAGCACGACUUCUUCAAGdTdT Pmn - dTdTCGUCGUGCUGAAGAAGUUC
	ss3´-Pmn/as3´-Pmn	GCAGCACGACUUCUUCAAGdTdT - Pmn Pmn - dTdTCGUCGUGCUGAAGAAGUUC
	ss/as3´-Biotin	GCAGCACGACUUCUUCAAGdTdT Biotin - dTdTCGUCGUGCUGAAGAAGUUC

Bulge-containing siRNA

ss/as-bulge

ss-bulge/as

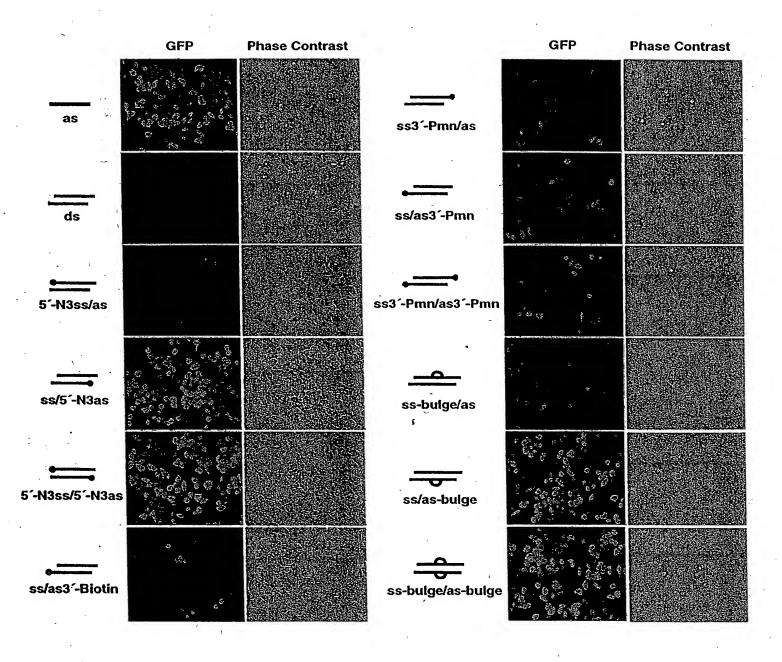
GCAGCACGA CUUCUUCAAGdTdT dTdTCGUCGUGCU GAAGAAGUUC CA

dTdTCGUCGUGCU GAAGAAGUUC

UG GCAGCACGA CUUCUUCAAGdTdT

ss-bulge/as-bulge

UG GCAGCACGA CUUCUUCAAGdTdT dTdTCGUCGUGCU GAAGAAGUUC CA



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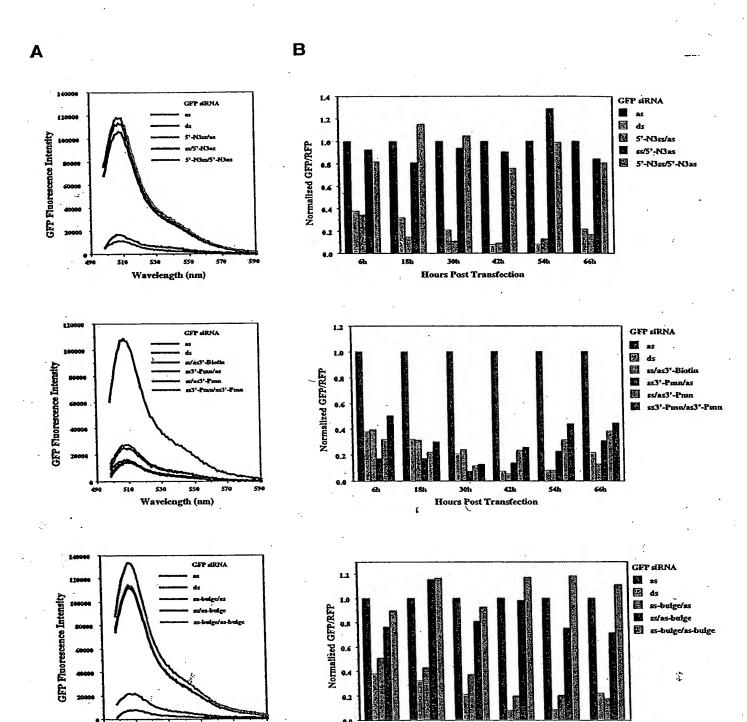
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Wavelength (nm)

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Hours Post Transfection



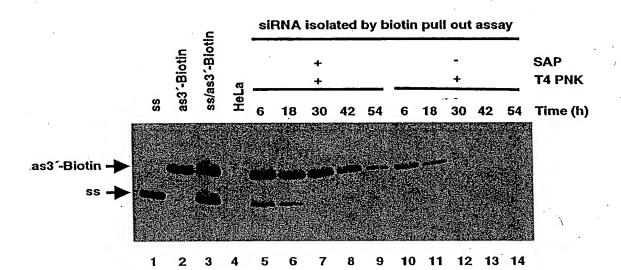
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Fig 5



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В

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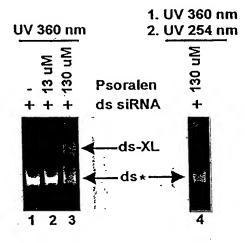
A Psoralen (HMT)

HO,

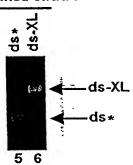
GFP ds siRNA

GCAGCACGACUUCUUCAAGdTdT dTdTCGUCGUGCUGAAGAAGUUC

C

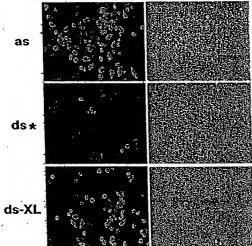


Purified siRNA

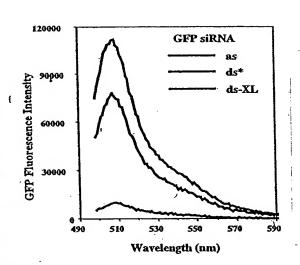


D





E

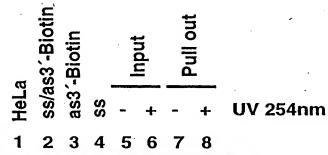


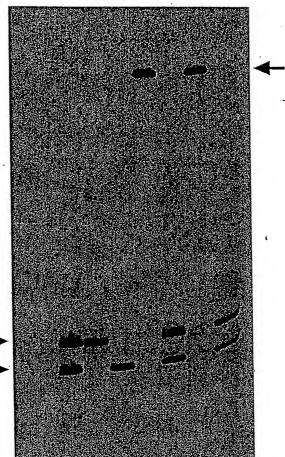
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Fig 7

ss/as3´-Biotin-XL





as3'-Biotin -

ss ·

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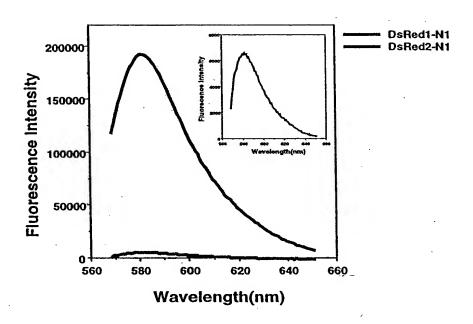
Title: IN VIVO GENE SILENCING BY CHEMICALLY MODIFIED

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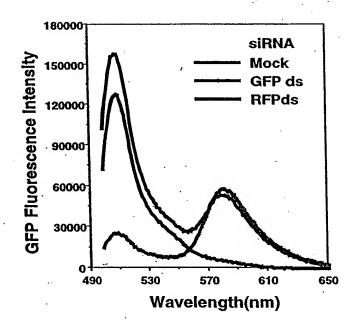
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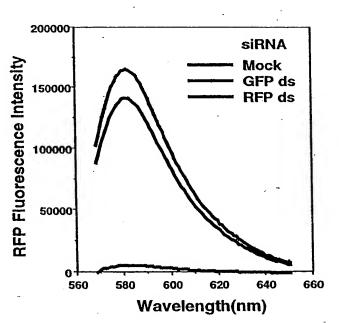
Fig 8

(A)



(B)



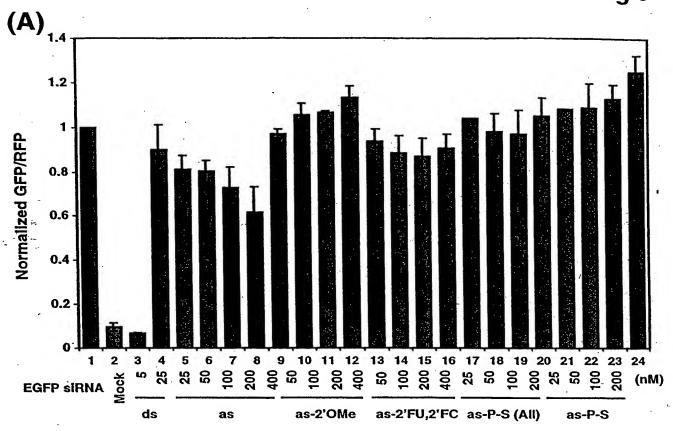


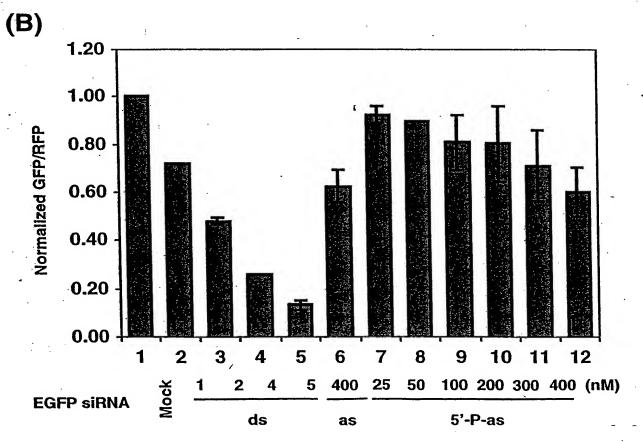
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Fig 9





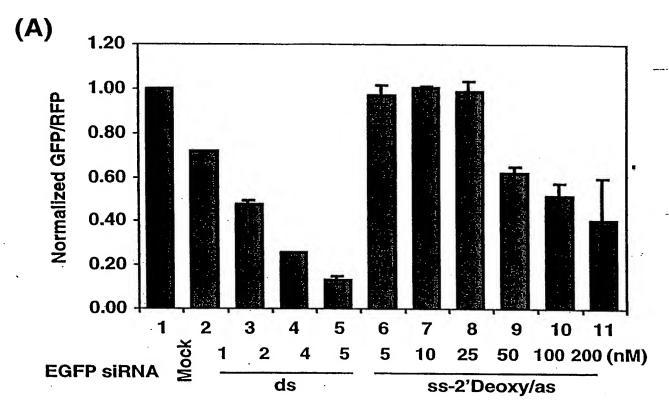
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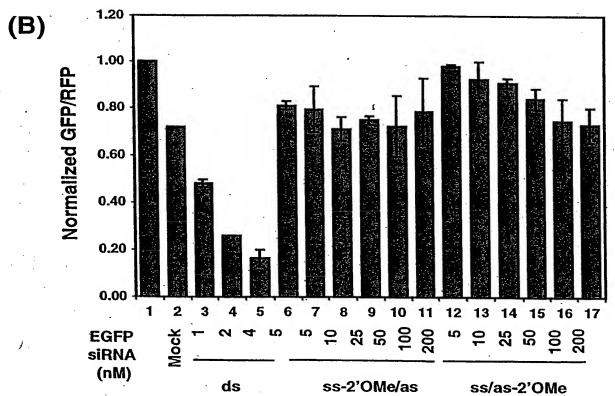
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Fig 10





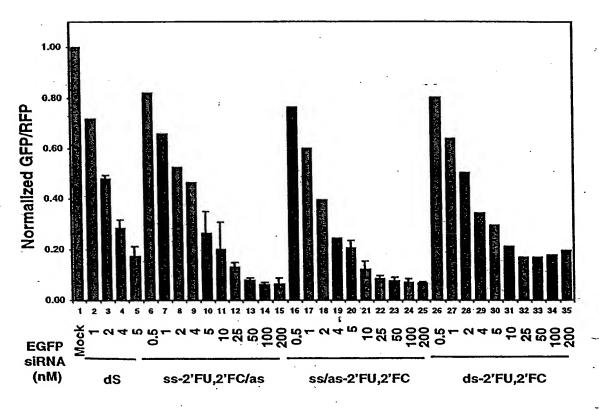
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Fig 10

(C)



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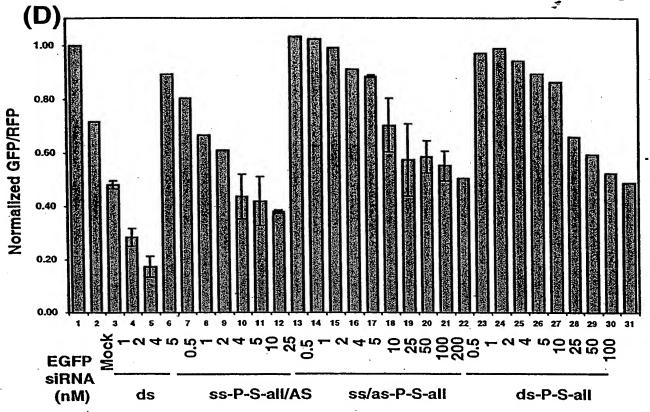
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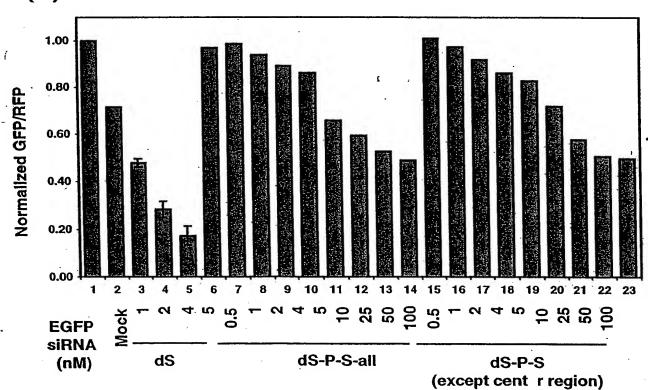
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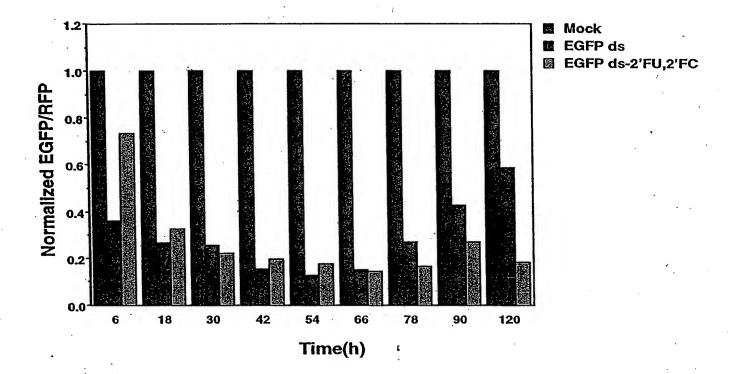




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: Fig 11



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Title: IN VIVO GENE SILENCING BY CHEMICALLY MODIFIED AND STABLE siRNA Sheet 16 of 26 (A) Antisense strand siBNA Unmodified = 100.0% 2'OMe 2'FU,2'FC PhorPhorothiolates(All) 80.0% **Phorphorothiolates** % of Intact sIRNA 60.0% 40.0% FIG 12A-C 20.0% 0.0% 10 Time (min) Duplex siRNA with modification ... at antisense strand (B 100.0% Unmodified . 2'OMe 2'FU,2'FC 80.0% Phosphorothiolates(All) Phosphorothiolates % of Intact siRNA 60.0% 40.0% 20.0% 0.0% 30 0 10 20 50 60 Time (min) i Duplex siRNA with modification at both strand 100.0% Unmodified 2'OMe 2'FU,2'FC 80.0% Phosphorothiolates(All) **Phosphorothiolates** % of Intact siRNA 60.0% 40.0% 20.0%

0.0%

10

20

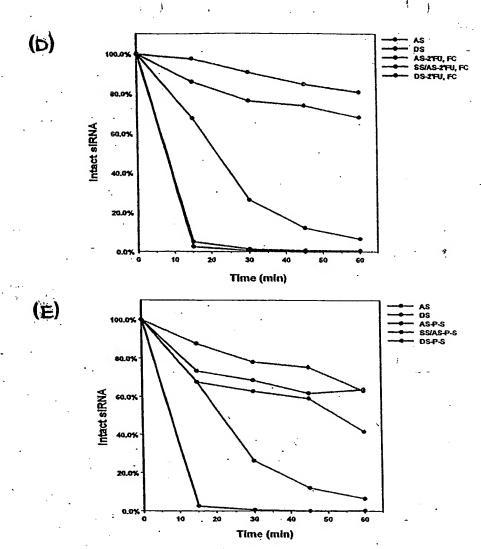
30

Time (min)

40

50

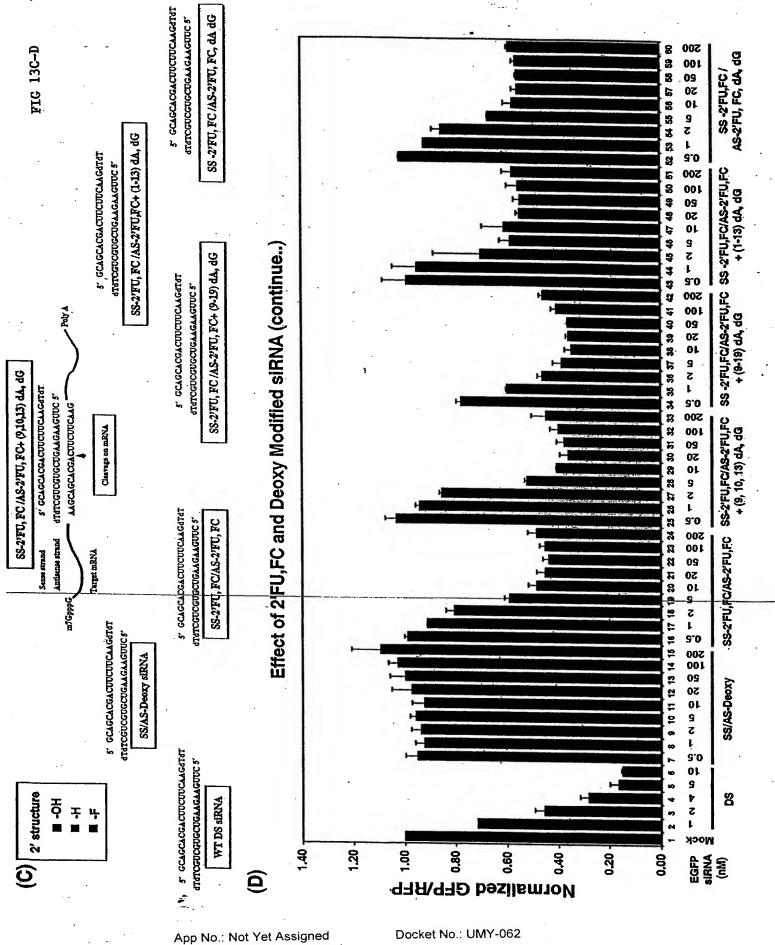
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Title: IN VIVO GENE S!LENCING BY CHEMICALLY MODIFIED AND STABLE siRNA Sheet 18 of 26 s' GCAGCACGACTUCTUCAAGATAT ATATOGUCCUGAAGAAGUUC 5' FIG 13A-B SS/AS-2'FU, FC, dA dG 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 14 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 62 53 54 55 56 57 58 59 60 200 SS/AS-2'FU, FC, dA, dG 100 20 01 200 0.6 0.6 5' GCAGCACGACUUCUUCAAGGTdT SS/AS-27FU,FC+ (1-13) dA, dG ATATCGUCGUGCUGAAGAAGUUC 5 100 SS/AS-2'FU,FC + 20 20 10 9 1 2 Effect of 2'-FU,FC and Deoxy Modified siRNA s' GCAGCACGACUUCUUCAAGUTAT SS/AS-2'FU, FC+ (9-19) dA, dG **500** ATATICETICETIGETIGAAGAAGUUC 5' 100 20 50 SS/AS-2'FU,FC + 200 0.6 1 2 2 SS/AS-2'FU, FC+ (9,10,13) dA, dG S' GCAGCACGACTUCTUCAAGATAT ATATCEUCEUGCUGAAGAAGUUC S' AAGCAGCACGACTUCTAGG 100 SS/AS-2'FU,FC+ 20 20 Cleavage on mRNA 10 0.5 1 2 2 s ccaccaccoccoccacatar STATCGUCGUGCUGAAGAAGUUC 5' 500 100 SS/AS-2'FU,FC Antisense strand SS/AS-2'FU, FC 20 10 20 farget mRNA Sense strand 2.0 1 2 m7GpppG S' GCAGCACGACTUCTUCAAGGTAT ATATCGUCGUGCUGAAGAAGUUCS' 200 20 20 20 10 20 10 SS/AS-Deoxy sfRNA SS/AS-Deoxy 3.0 S' GCAGCACGACUUCUAAGATAT 1 2 4 5 dtatoguegegearaaaguuc 5' 8 2' structure WT DS sIRNA 주 두 MOCK 1.20 99. 0,80 0.60 0.40 0.20 5 0.0 SIRNA (nM) Normalized GFP/RFP

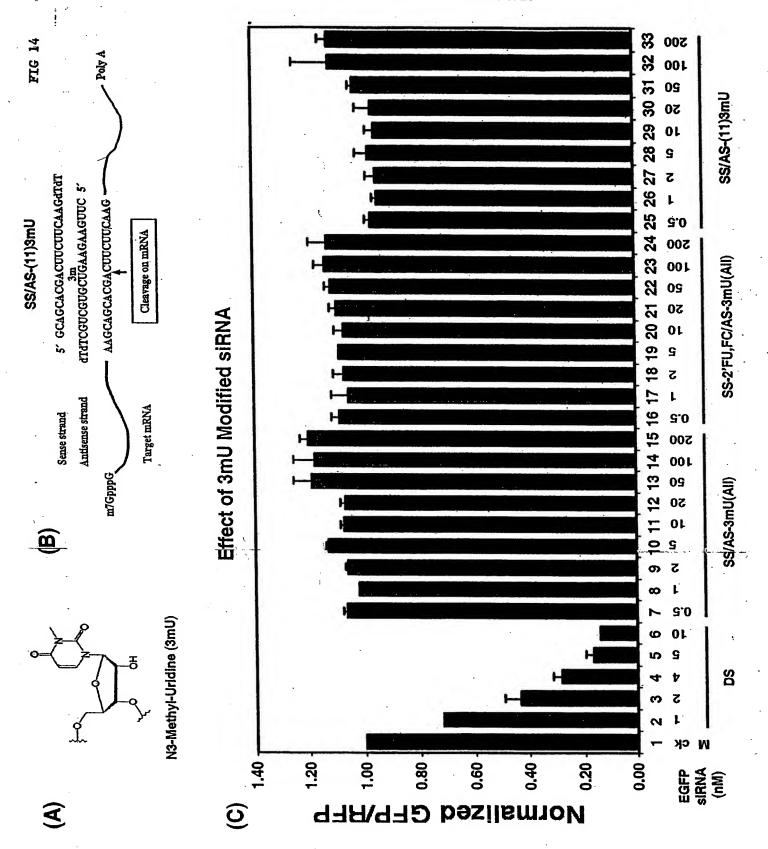


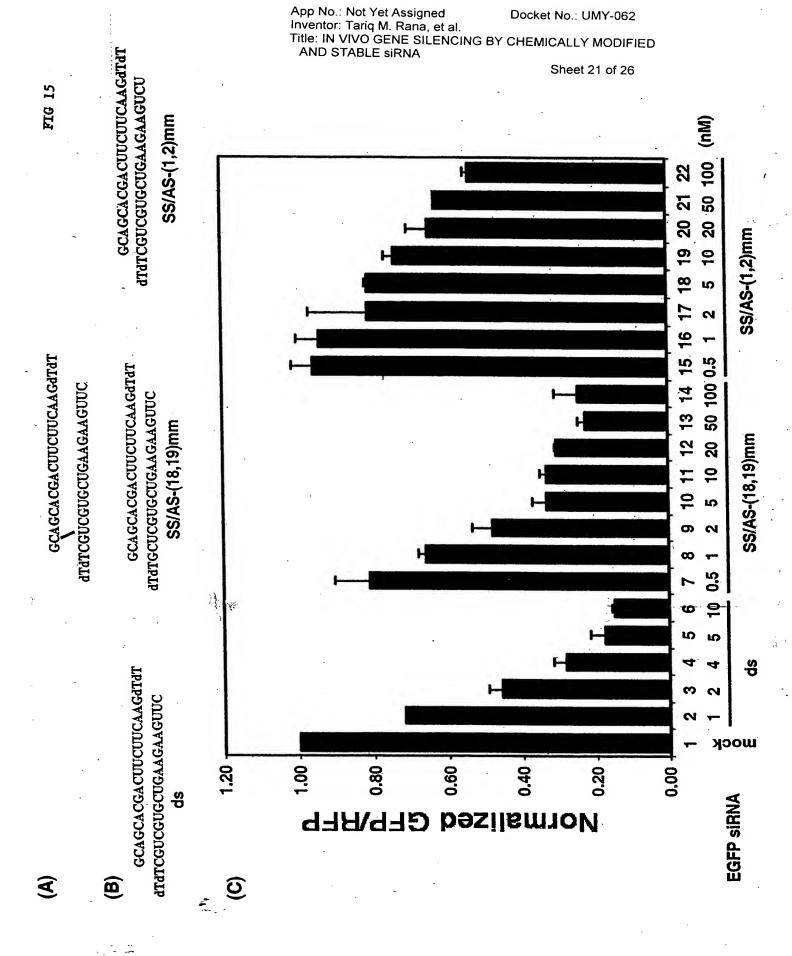
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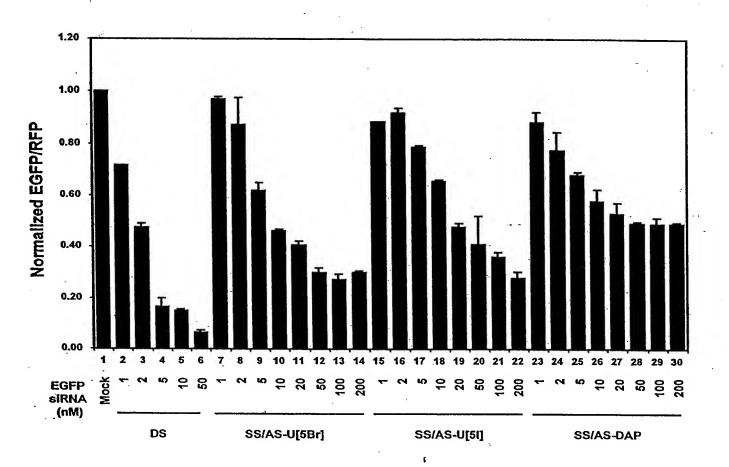
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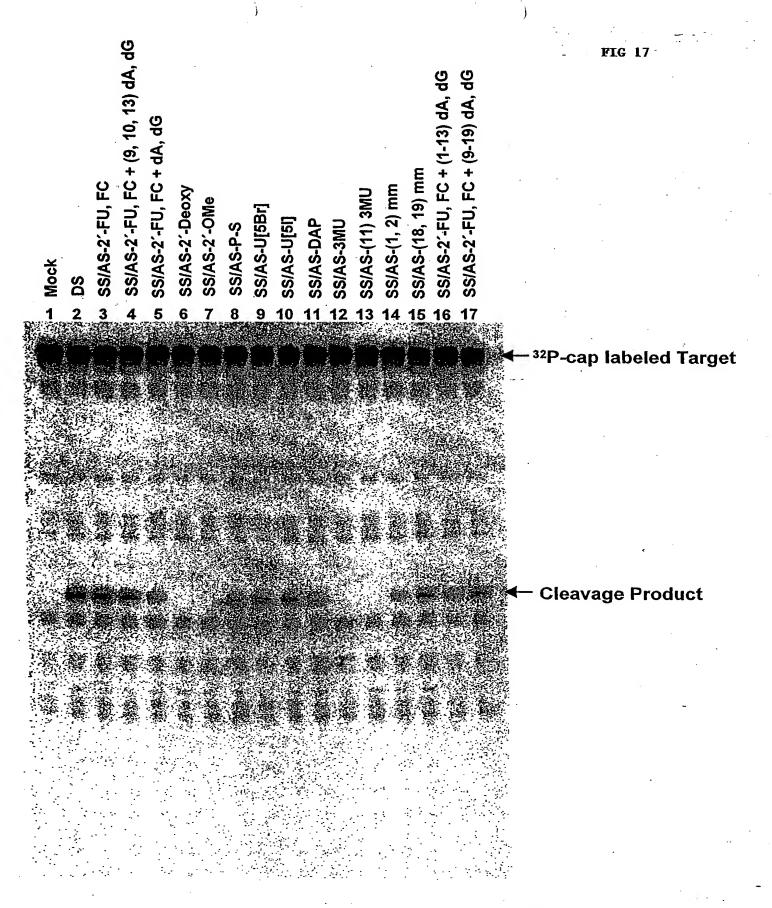
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FIG 16





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5-Bromo-uridine = U[5Br]

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OH

5-lodo-uridine = U[51]

AND STABLE SIRNA

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OH

2,6-Diaminopurine = DAP

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Photocleavable Biotin